the distal carboxylate group is disposed opposite to the methyl cap to escape the steric repulsion from the cap. Consequently, Δ-[Co(sen)]$^{3+}$ forms a relatively stable ion pair with the [Sb_2-(d-tart)$_2$]$^{2-}$ ion in the C_2 association like Δ-[Co(X)$_2$(en)$_2$]$^+$-type complexes. In this way, our C_3 and C_2 association models afford a reasonable interpretation to the novel CD spectral changes of Λ- and Δ-[Co(sen)]$^{3+}$ shown in Figure 4.

Finally, it is noteworthy that the DCD spectrum[6,10,13] of Λ-[Co(sen)]$^{3+}$ is much smaller in magnitude than that of Δ-[Co-(sen)]$^{3+}$ and than those of Λ- and Δ-[Co(sep)]$^{3+}$ and of Λ- and Δ-[Co(chxn)$_3$]$^{3+}$ for which only either the C_2 or the C_3 association is sterically allowed. This is interpreted to mean that Λ-[Co-(sen)]$^{3+}$ adopts not only the C_3 but also the C_2 association mode in the interaction with the [Sb_2(d-tart)$_2$]$^{2-}$ ion, and that the CD change due to the C_3 association is cancelled out by the opposite CD change due to the concomitant C_2 association because of a small energy difference between the A_2 and E_a states for [Co-(N)$_6$]$^{3+}$-type complexes.[14] In fact, the steric repulsion imposed by Λ-[Co(sen)]$^{3+}$ on the chiral anion in the C_2 association is not so severe, since it is the same in nature as that imposed by Λ-[Co(sep)]$^{3+}$, which forms a relatively stable ion pair with the chiral anion in the C_2 association (see the left-hand models in Figures 2 and 5). Thus, it is fairly probable that Λ-[Co(sen)]$^{3+}$ adopts both the C_3 and C_2 modes in the interaction with the [Sb_2(d-tart)$_2$]$^{2-}$ ion. Then, the association constant K_A of Λ-[Co(sen)]$^{3+}$ derived from the usual analysis of the CD changes[6] is artificial and is almost meaningless, since two different association modes are present between Λ-[Co(sen)]$^{3+}$ and the [Sb_2(d-tart)$_2$]$^{2-}$ ion.[15] In fact, the α value of [Co(sen)]$^{3+}$ derived by HPLC is not as high as expected from the apparent K_A values of the two enantiomers.[6]

Similarly, relatively small DCD spectra observed for Λ- and Δ-[Co(en)$_3$]$^{3+}$ [6,10] point to the presence of the concomitant C_2 association even for [Co(en)$_3$]$^{3+}$,[16] though its chirality is recognized mainly by the C_3 association. Then, if [Co(en)$_3$]$^{3+}$ were forced to adopt the C_3 association mode only, its chirality would be recognized more effectively by [Sb_2(d-tart)$_2$]$^{2-}$ ion in solution.

One naive question arises as to why the C_2 association does not predominate in Δ-[Co(en)$_3$]$^{3+}$, but it does in Δ-[Co(sen)]$^{3+}$. This question is answered in terms of two structural differences between [Co(en)$_3$]$^{3+}$ and [Co(sen)]$^{3+}$. The first is that for the former complex, both sides of the C_3 axis are available for the C_3 hydrogen-bonding association with the [Sb_2(d-tart)$_2$]$^{2-}$ ion, while only one side is available for [Co(sen)]$^{3+}$. Therefore, [Co(en)$_3$]$^{3+}$ tends to adopt the C_3 mode more frequently than [Co(sen)]$^{3+}$. Another difference is that for [Co(sen)]$^{3+}$, a secondary amine proton is involved in the C_2 hydrogen-bonding interaction with the [Sb_2(d-tart)$_2$]$^{2-}$ ion, while it is not involved at all for [Co-(en)$_3$]$^{3+}$. Since a stronger hydrogen bond is formed with a secondary amine proton than with a primary one,[1,8,17] the stronger C_2 association with the [Sb_2(d-tart)$_2$]$^{2-}$ ion is naturally expected for [Co(sen)]$^{3+}$. By contrast, only primary amine protons are involved in the C_3 association for both complexes.

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Reaction of the Antitumor Drug *cis*-Diamminedichloroplatinum(II) with the Trinucleotide d(GpApG): Identification of the Two Main Products and Kinetic Aspects of Their Formation

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The two main products (total yield over 95%) that are obtained in the reaction of *cis*-diamminedichloroplatinum(II) with the trinucleotide d(GpApG) have been characterized by high-field proton NMR (300 and 500 MHz; using the pH dependence of the chemical shift of the nonexchangeable base proton signals as well as homodecoupling and NOE techniques) and by the analysis of their enzymatically digested products with anion-exchange chromatography (FPLC) and platinum atomic absorption spectroscopy. The results indicate the formation of both *cis*-Pt(NH$_3$)$_2$[d(GpApG)-*N*7(1),*N*7(3)] (yield 80%) and *cis*-Pt(NH$_3$)$_2$[d-(GpApG)-*N*7(2),*N*7(3)] (yield 20%). No influence due to temperature or prior hydrolysis of *cis*-PtCl$_2$(NH$_3$)$_2$ was observed on the product ratio. The reaction of d(GpApG) with the monofunctional platinum compound [PtCl(dien)]Cl, mimicking the first binding step of *cis*-PtCl$_2$(NH$_3$)$_2$, provides more insight into the overall chelate formation. The observation that only an AG chelate but no GA chelate is formed agrees with other studies in which also only the AG chelate is reported. This can be explained by the geometry of the trinucleotide.

Introduction

The working mechanism of the widely applied antitumor drug *cis*-PtCl$_2$(NH$_3$)$_2$ (*cis*-diamminedichloroplatinum(II), abbreviated as *cis*-Pt) and structurally related compounds is only partly understood.[1] Nevertheless, many indications, mainly from biochemical studies, led to an almost general acceptance of the idea that the interaction of *cis*-Pt with cellular DNA is the most important event in the working mechanism of this drug.[1-3] This hypothesis focused many chemical, biochemical, and biophysical studies on the interaction of certain platinum coordination

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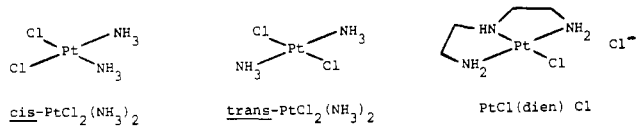

Figure 1. Schematic structures of the antitumor active cis isomer and the inactive trans isomer of $PtCl_2(NH_3)_2$ and monofunctional [PtCl(dien)]Cl.

compounds—such as the bifunctional *cis*-Pt, its inactive trans isomer and monofunctional [PtCl(dien)]Cl (see Figure 1)—with DNA and DNA fragments, e.g. mononucleotides or oligonucleotides with well-defined sequences.[2,4]

Competition studies revealed that these platinum compounds have a strong kinetic preference for the nucleobase guanine.[4,5] This led to the idea that the first reaction of bifunctional *cis*-Pt and DNA occurs with this nucleobase. The second interaction can result in various bifunctional chelates, depending on the local DNA sequence and the DNA conformation around the initially bound guanine. It is not known, however, which of these adducts represents the "key-lesion", to which the observed cytotoxic activity can be ascribed.

From "in vitro" studies, it has become clear that in addition to a monofunctional adduct, at least four bifunctional platinum–DNA adducts can be formed: the GG chelate (most abundant), the AG chelate, an adduct in which *cis*-Pt is bound to two guanines positioned in the complementary DNA strands, and the so-called GNG chelates[6]. The latter binding mode—in which *cis*-Pt is attached to two next-neighboring guanines with a noncoordinating base (N) in between—was first deduced from the results of a bacterial mutation study.[7] This binding fashion was afterward also established in short oligonucleotides, such as d(GpCpG)[8] and d(GpTpG),[9] as well as in an d(GpTpG)-containing undecamer.[10]

From a kinetic point of view, the interaction of *cis*-Pt with the GNG trimers d(GpApG) and d(GpGpG) is especially interesting, since in these sequences both neighboring and next-neighboring purines can coordinate to platinum. In this paper a study on the interaction of *cis*-Pt with the trinucleotide d(GpApG) (abbreviated GAG) is presented. It is concluded that in this reaction *cis*-$Pt(NH_3)_2$[d(GpApG)-*N*7(1),*N*7(3)] (80%; abbreviated *GAG*) and *cis*-$Pt(NH_3)_2$[d(GpApG)-*N*7(2),*N*7(3)] (20%; abbreviated G*AG*) are formed. No indications for *GAG* or for a product in which adenine N1 is involved in the *cis*-Pt chelation are found. The identification of both adducts is based upon proton NMR techniques and upon analysis of their enzymatically digested products by anion-exchange chromatography (FPLC) in combination with platinum atomic absorption spectroscopy (AAS). The kinetics of the chelate formation leading to both adducts was studied in more detail by simulating the first platination step with the monofunctional [PtCl(dien)]Cl.

Materials and Methods

Starting Materials. The platinum compounds *cis*-$PtCl_2(NH_3)_2$ and [PtCl(dien)]Cl were prepared as published elsewhere.[11,12] *cis*-Pt was purified by crystallization from DMF.[13] d(GpApG) was synthesized according to an improved phosphotriester method.[14]

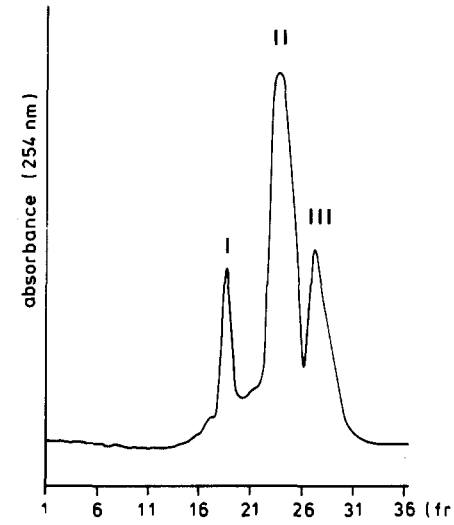

Figure 2. Gel permeation (Sephadex G25; Pharmacia) elution profile of the reaction mixture containing an equimolar amount of GAG and *cis*-Pt, after completeness of the reaction.

Reactions and Purification of the Adducts. A solution containing equimolar amounts of GAG and *cis*-Pt, (about 10^{-5} M; assuming a molar absorption of 34 000 M^{-1} cm^{-1} for GAG at 254 nm and pH 7.0) was incubated for 14 days in the dark at room temperature. Due to reaction, the UV absorption maximum and minimum values of the solution increased from 254 to 259 nm and from 222 to 231 nm, respectively. The max/min ratio of the UV absorption decreased during these 2 weeks from 4.2 to a constant value of 2.1, which indicated completion of the reaction. Separation of the two products and unreacted GAG (10%) was achieved by gel permeation chromatography (Sephadex G25; Pharmacia) using 0.02 M triethylammonium hydrogen carbonate (TEAB) as eluent. The reaction of the trinucleotide with [PtCl(dien)]Cl was performed at 25 and 50 °C under similar conditions as described for the reaction with *cis*-Pt. The platinum compound was added when the desired temperature was achieved in the GAG solutions.

NMR Sampling. The TEAB in the product fractions was removed by repeated evaporation. The compounds were lyophylized two times from D_2O (99.8%, Merck). A trace of tetramethylammonium nitrate (TMA) was added as internal reference (3.18 ppm downfield from DSS). After pH adjustment, the samples were lyophylized again and dissolved in 0.4 mL of D_2O (99.95%, Merck).

NMR Measurements. The proton NMR spectra were recorded at 300 and 500 MHz on Bruker WM 300 (pH plots) and Bruker WM 500 (homodecoupling and NOE experiments) instruments, both interfaced with an Aspect 2000 computer. Resolution enhancement was performed by applying a Gaussian window, according to Ernst.[15] The pH dependence of the chemical shifts of the nonexchangeable base protons was monitored by adding traces of DCl and NaOD. The pH values are not corrected for isotope effect. If necessary, the HDO signal was reduced by selective saturation.[16] Homodecoupling and 1D NOE experiments (mixing time, 0.3 s) using the WEFT[17] and the DANTE[18] pulse sequence, respectively, to reduce the HDO-signal were performed at 4 °C (GAG and *GAG*) or at 11 °C (*GAG*). The samples were repeatedly degassed under nitrogen atmosphere to obtain optimal NOE effects.

Effect of Temperature and Prior *cis*-Pt Hydrolysis on the Adduct Ratio. For these studies, 1.0 mL of GAG solution (A_{254} = 10; pH 7.0) was incubated in the dark with 0.5 equiv of either *cis*-Pt (final NaCl concentration in the solution 4 mM) or *cis*-$Pt(OH)(H_2O)(NH_3)_2^+$ (without NaCl). The reactions were performed at 37 (7 days and 1 day, respectively), 50 (2 days and 1 day, respectively) or 80 °C (6 and 1 h, respectively). Under these conditions, the reactions appeared to be complete. A longer incubation time at 80 °C appeared to result in degradation of the trinucleotide. *cis*-$Pt(OH)(H_2O)(NH_3)_2^+$ was obtained from *cis*-$PtI_2(NH_3)_2$ after the latter was stirred for 2 h with 2 equiv of $AgNO_3$. Afterwards, the pH of the sample was adjusted to 7.0 with a NaOH solution. AgI was removed by centrifugation. The reaction

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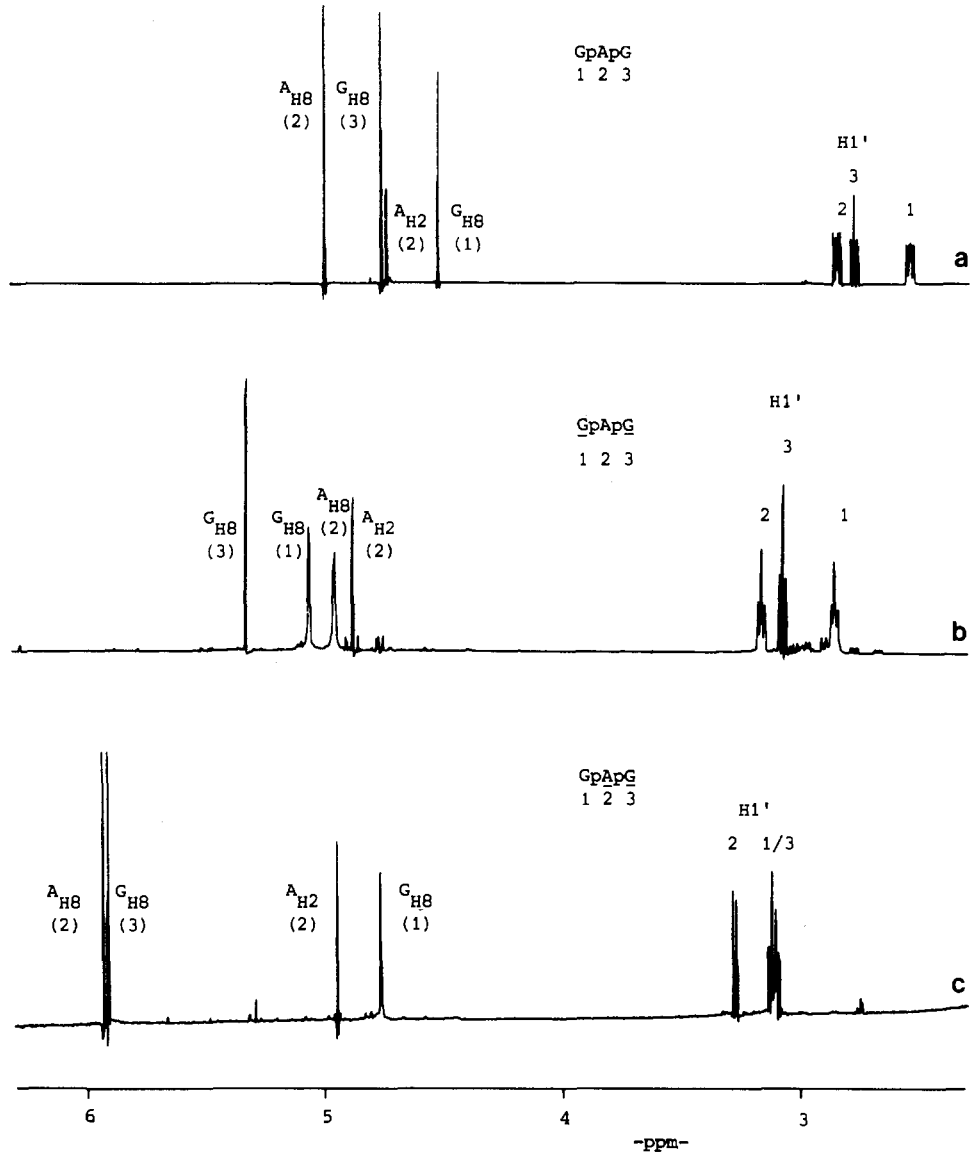

Figure 3. Downfield region of the 500-MHz proton NMR spectrum of unreacted d(GpApG) (a), the major product (b), and the minor product (c). The spectra are recorded at 4 °C, with TMA as internal reference.

products were separated by anion-exchange chromatography (FPLC).

Enzymatic Digestions. Both *cis*-Pt adducts were digested with deoxyribonuclease-1 (Sigma) and nuclease P1 (Sigma) for 16 h at 37 °C as described previously.[6] The formed products were separated by FPLC.

Fast Protein Liquid Chromatography (FPLC). FPLC separation of the trinucleotide adducts, as well as their digestion products, was achieved by anion-exchange chromatography on an FPLC system (Pharmacia), equipped with a Mono Q column (Pharmacia), a 254-nm UV detector (Pharmacia), and a Spectra Physics SP4100 integrating system. Details, such as buffers and used gradients, have been described by Fichtinger-Schepman et al.[6]

Atomic Absorption Spectroscopy (AAS). To determine the platinum amount in the fractions obtained by the FPLC separation, 0.5-mL fractions were collected and monitored for the metal by AAS on a Perkin-Elmer Model 4000 instrument, connected to an HGA-500 graphite furnace and an AS-400 autosampling system. Calibration was performed with K_2PtCl_6 solutions.

Nomenclature. The notation of the chain direction is according to the recommendations by the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN); i.e., the oligonucleotide units are notated sequentially from the left to the right from the 5′- to the 3′-terminus.[19]

Results and Discussion

General Observations. After the equimolar reaction between GAG and *cis*-Pt, gel permeation chromatography resulted in the separation of three UV-absorbing compounds (see Figure 2). The downfield parts of their proton NMR spectra are redrawn in Figure 3. As expected, four base proton singlets (two H8 signals of the guanines and one H2 signal and one H8 signal of adenine) and three H1′ signals are present around 5 and 3 ppm, respectively. The first eluted fraction (I) appeared to contain unreacted trinucleotide, since an identical NMR spectrum and the same UV max/min ratio was found as for the starting material. The other two product fractions (II and III) contained *cis*-Pt-bound trinucleotide, as deduced from the downfield shifts of some base proton signals, which are known to originate from platinum binding.[4] Moreover, an apparent alteration in the UV spectrum, such as a bathochromic shift and a decrease in the max/min ratio confirmed this.

To assign the coordination sites of *cis*-Pt to the trinucleotide in both products, three independent approaches have been applied:

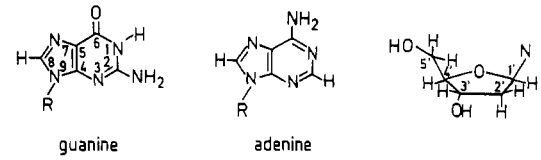

Figure 4. Structure and numbering scheme of the nucleobases adenine and guanine and of the *d*-ribose (R) residue, to which the nucleobases (N) are attached.

(19) IUPAC-IUB Joint Commission on Biochemical Nomenclature, Recommendations: *Eur. J. Bioch.* **1982**, *131*, 9.

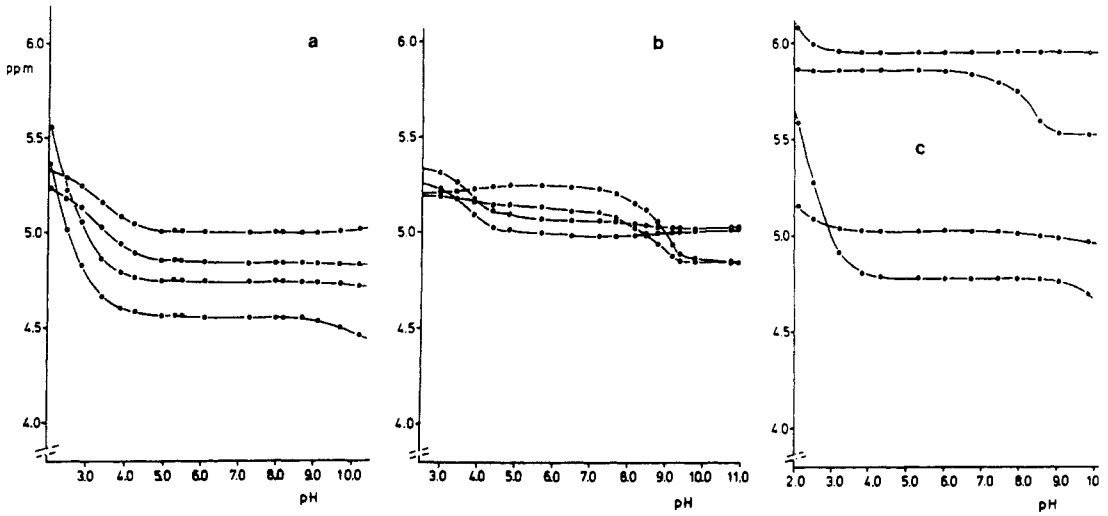

Figure 5. Plots showing the pH dependence of the chemical shifts of the nonexchangeable base protons in unbound d(GpApG) (a), the major product (b), and the minor product (c), monitored at 57 °C.

(a) monitoring the pH dependence of the chemical shift of the base proton signals; (b) decoupling and NOE experiments; (c) analysis of the enzymatically digested products of the adducts.

pH Dependence of the Nonexchangeable Base Protons. In Figure 4, the nucleobases guanine and adenine and the deoxyribose residue with their numbering schemes are depicted. The chemical shift of the nonexchangeable base protons (guanine H8 and adenine H2 and H8) is influenced by N1 and N7 (de)protonations. N7 protonation usually gives rise to relatively large changes in chemical shift of the H8 proton resonance (about 1 ppm in mononucleotides), while N1 (de)protonation is known to cause a smaller shift (about 0.3 ppm).[20] Platination of these sites prevents protonation. Monitoring the pH dependence of the chemical shift of the four base protons can therefore supply direct information about the coordination sites of *cis*-Pt in GAG.

Figure 5 shows the pH profiles of unreacted d(GpApG) (a), the major product (b), and the minor product (c). In Figure 5a, the large protonation effect on two curves observed around pH 2 results from N7 protonation of both guanine residues. The expected corresponding N1 deprotonation effect (around pH 10) is hardly seen on these H8 signals. The two remaining curves must belong to adenine. The N1 protonation effect, observed around pH 3, confirms this. The adenine N7 protonation is known[21] to occur below pH 0.

In the pH plot of the major product (Figure 5b), four curves can be distinguished, which can be grouped into two sets of two. One set shows an N1 deprotonation effect around pH 8.5 but no N7 protonation effect. This is a clear indication for N7 platination of guanines residues.[22] The curvature of the remaining signals is essentially the same compared to that of the adenine signals in the unreacted trinucleotide; the pK_a of the adenine N1 protonation remains 3. Undoubtedly, *cis*-Pt is N7 bound to both guanines in the main product.

In the pH plot of the minor product (Figure 5c), the curves belonging to one unbound and one N7-platinated guanine can be easily distinguished (vide supra). The remaining two adenine signals both show a decrease of the pK_a of the N1 protonation from pH 3 to 2 while also a large downfield shift on one of these signals is observed. This clearly points to adenine N7 platination. Adenine N1 platination can be excluded, because in that case no protonation would occur in the pH range used.[23] One should be aware of the fact that from these data alone it is impossible to conclude unambiguously which of the two guanines is involved in the platinum binding. So, at this stage, we cannot yet decide between GA and AG chelation.

It is furthermore interesting to note that in the NMR spectrum of the minor product an H1′ doublet splitting pattern is observed (see Figure 3c), which is not present in the spectra of GAG and the major product. Several studies dealing with N7 chelation of neighboring purines by *cis*-Pt have made clear that this binding is accompanied by the almost complete disappearance of the H1′–H2′ coupling in the *d*-ribose ring connected to the 5′-coordinated purine.[24] This phenomenon has been explained by an almost pure N-type conformation of this sugar residue.[25] In general, *d*-riboses adopting an almost pure N conformation can be identified by the appearance of H1′ doublet splitting patterns in the NMR spectrum, whereas in the case of S-type conformers (most common in DNA fragments[26]), triplets or quartets are to be expected. In our case, the appearance of an H1′ doublet in the minor product is in nice agreement with the above-proposed *cis*-Pt chelate of two neighboring purines. In *GAG*, this N-type sugar conformation is not observed, possibly due to the greater flexibility in GNG-chelates. The low-field position of the H1′ doublet in the minor product suggests a 5′-bound adenine, since the H1′ signal of adenine is usually found more downfield than that for the guanine H1′. As only the ribose moiety of the 5′-platinated purine adopts an N-type conformation, this would implicate that the minor product should be the AG chelate.

Homodecoupling and NOE Techniques. In this approach, the identity of the nonexchangeable nucleobasebase proton signals in the spectra of the unbound trinucleotide and both *cis*-Pt adducts can be determined in principle by straightforward assignment of these protons to the proper sugar residue. This, in combination with the results of the above-mentioned pH plots, can afford an unambiguous assignment of the trinucleotide residues to which platinum is bound. Therefore, first scalar-coupled sugar protons were assigned to a sugar residue by extensive 1D-homodecoupling experiments, starting with the characteristic high-field position of the H5′/H5″ signals belonging to the 5′-end sugar and the characteristic shape of the H3′ signal belonging to the 3′-end sugar.[26] The base protons were subsequently connected to the proper sugar residue by 1D-NOE difference experiments.

Figure 6 shows the NMR spectrum of the major product with the complete assignments of the three sugar residues and the nonexchangeable base protons as well as three NOE-difference

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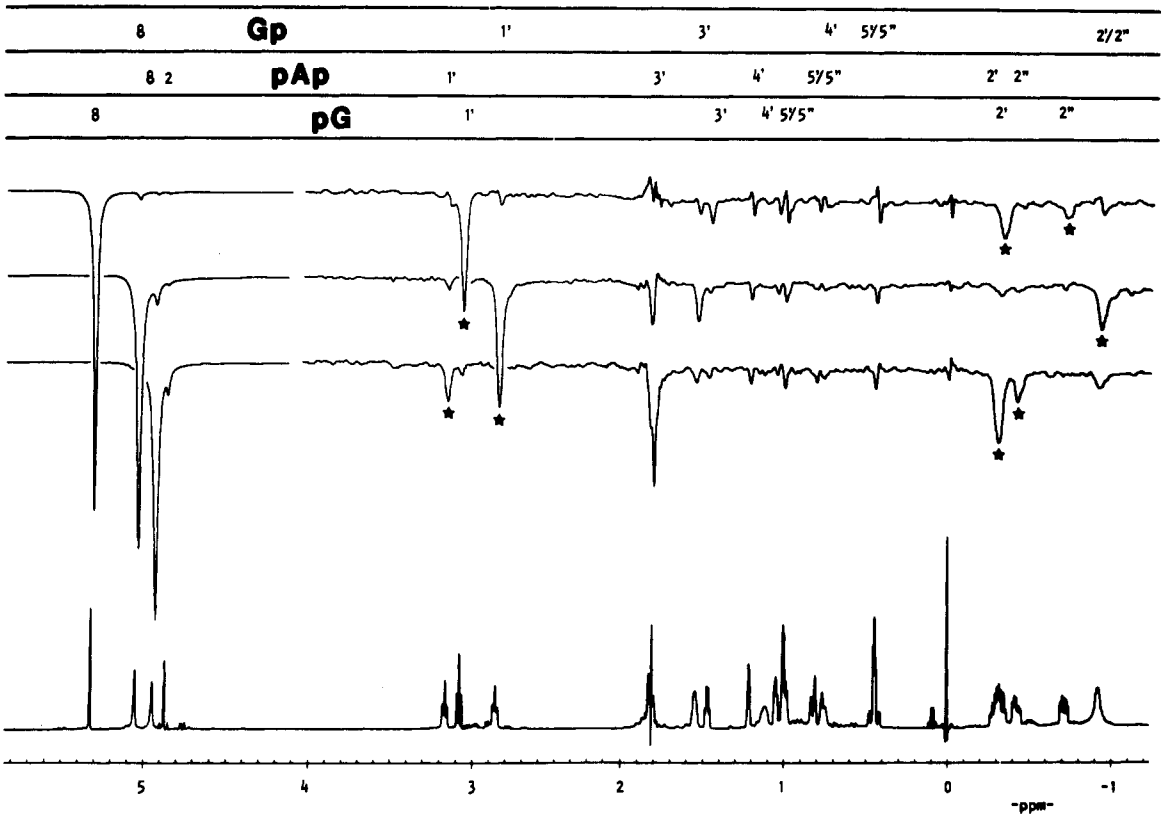

Figure 6. Proton NMR spectrum (500 MHz) of *GAG*, recorded at 4 °C with complete assignment of the sugar and base protons signals, and the NOE difference spectra, obtained after irradiation of the three H8 protons.

spectra obtained after irradiation of the three H8 protons. Clear NOE effects of these purine signals on the corresponding *d*-ribose via H1′ and H2′/H2″ are indicated. In this way, the base protons of the major product and the unbound trinucleotide could be assigned unambiguously. Although the NOE effects in the minor adduct were much smaller, it was possible to link the unbound guanine H8 signal (upfield; see Figure 3c) to the 5′-terminus of the trinucleotide. This proves the identity of the minor product as the AG chelate, in agreement with the above-mentioned tentative assignment based upon chemical shift considerations.

The homodecoupling and NOE results eventually led to the assignment depicted in Figure 3. Discrimination between the H8 and the H2 of adenine in the unbound trinucleotide and the major product was performed by H8-exchange experiments, based upon the acidic character of the H8 in purines,[27] in which this proton is substituted for deuterium (data not shown).

Enzymatic Digestion of the Two Adducts Followed by Anion-Exchange Chromatography and Platinum Measurements. The third approach to characterize the two products was performed by the analysis of their enzymatic digestion products. In previous studies, it has been demonstrated that DNA treated with *cis*-Pt, and also platinated oligonucleotides, can be degraded by deoxyribonuclease 1 and nuclease P1 to 5′-mononucleotides and platinum adducts.[6] The phosphodiester bridge between neighboring nucleotides bound by *cis*-Pt remains intact, while adducts in which platinum is bound to guanines with a third base (N) in between (next-neighboring bound bases) are degraded to (5′-dNMP)- and $(5'\text{-dGMP})_2\text{Pt(II)}$ adducts.

Digestion of the major product (*GAG*) should therefore yield 5′-dAMP and *cis*-$\text{Pt(NH}_3)_2$(dGuo-*N*7)(5′-dGMP-*N*7). Digestion of the minor product should yield either *cis*-$\text{Pt(NH}_3)_2$[d-(GpA)-*N*7(1),*N*7(2)] and 5′-dGMP in the case of the GA chelate, or *cis*-$\text{Pt(NH}_3)_2$[d(pApG)-*N*7(1),*N*7(2)] and *d*-guanosine in the case of the AG chelate. Parts a and b of Figure 7 show the FPLC plots of both the UV absorbance and the platinum AAS measurements during the separation of the digested major and minor product, respectively. As expected, the platinum-containing fraction of digested *GAG* coeluted with synthetic[28] *cis*-$\text{Pt(NH}_3)_2$(dGuo-*N*7)(5′-dGMP-*N*7), and the other peak coeluted with 5′-dAMP. The identity of the platinum-containing peak of the digested minor adduct appeared to be *cis*-$\text{Pt(NH}_3)_2$[d-(pApG)-*N*7(1),*N*7(2)], which was also proven by coelution with the synthetic product. The elution time of the other peak is indicative for nucleosides such as *d*-guanosine. No 5′-dGMP

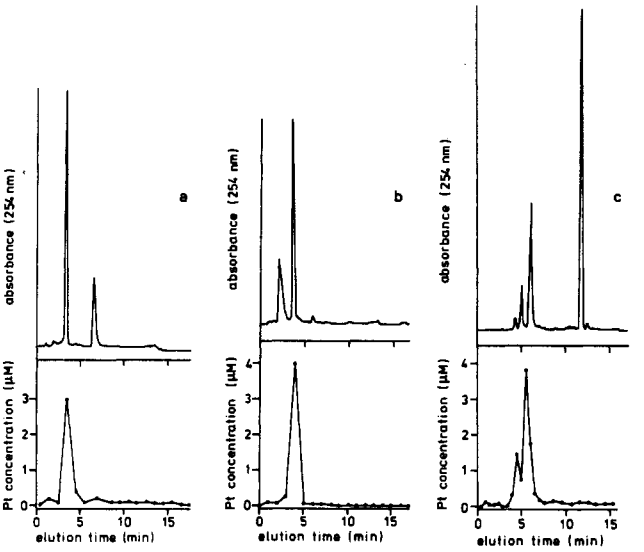

Figure 7. FPLC elution profiles of the UV absorbance and the corresponding platinum AAS data of the enzymatically digested *GAG* (a), the enzymatically digested G*AG* (b) and of a reaction mixture of *cis*-Pt and GAG without subsequent enzymatic digestion (c).

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(elution time: 12.3 min) was observed. This unambiguously proved the identity of the minor product to be *cis*-$Pt(NH_3)_2$[d-[GpApG)-*N*7(2),*N*7(3)], (*GAG*), in nice agreement with the results of the homodecoupling and NOE difference measurements.

Effect of Temperature and *cis*-Pt Hydrolysis on the Adduct Formation. Until now, almost all *cis*-Pt interactions with oligonucleotides were performed with the ultimate purpose to obtain one well-defined, platinated main product.[2,4] Therefore, the DNA fragments used were designed carefully to prevent appreciable amounts of "side products". For that reason, no kinetic studies are reported on *cis*-Pt interactions toward larger oligonucleotides, containing sequences with comparable reactivity. In this respect, d(GpApG) seems to be very suitable to start with, since two *cis*-Pt adducts are formed with this trinucleotide in good yield.

It can be expected that changing the reaction conditions can alter the relative amounts of the two adducts. Two important factors that can influence the adduct ratio are the temperature and the degree of the *cis*-Pt hydrolysis. First, the temperature can have a great influence on this ratio, since raising the temperature can strongly alter the conformation of the trinucleotide[26]. Second, the degree of the hydrolysis of *cis*-Pt can also have an important influence on the reaction velocity and on the formation of different amounts of adducts, because different reactivities have been reported for different *cis*-Pt hydrolysis products.

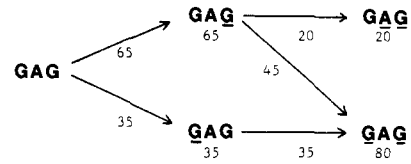
When *cis*-Pt is dissolved in water, a hydrolysis pathway—which depends on the chloride concentration and on the pH—takes place, leading to several hydrolyzed species. This *cis*-Pt hydrolysis appears to be a prerequisite for reactivity. Only the aqua species (i.e. the compounds in which at least one water molecule is coordinated to platinum) are considered to be reactive. So, *cis*-$PtCl_2(NH_3)_2$ and *cis*-$Pt(OH)_2(NH_3)_2$ are believed to be inactive.[29] When *cis*-Pt is dissolved in a 4 mM NaCl solution (to mimic intracellular conditions), *cis*-$Pt(OH)(H_2O)(NH_3)_2^+$ and *cis*-$PtCl(H_2O)(NH_3)_2^+$ appear to be the major aqua products at pH 7 and under equilibrium conditions that are present in the solution in a 5:2 ratio.[30] These aqua species might have a different preference toward the trinucleotide, leading to a different product ratio. This difference in reactivity can be monitored by comparing the reactivity of the above-mentioned hydrolysis solution of *cis*-Pt with a solution containing only *cis*-$Pt(OH)(H_2O)(NH_3)_2^+$ as the reactive aqua species. The latter species can be obtained from *cis*-$PtI_2(NH_3)_2$ by removing the I^- ligands with $AgNO_3$. Differences in reactivity between both aqua species have been reported implicitly.[31]

To identify and quantify the two trinucleotide adducts formed under the different reaction conditions, an anion-exchange chromatography separation (FPLC) was developed (see Figure 7c). Studies with the previously identified trinucleotides established the elution time of GAG (11.6 min), *GAG* (5.9 min), and G*AG* (4.8 min). A minute amount of product eluted at 3.8 min could not be characterized. This product was not separated by gel permeation (vide supra); however, its maximal yield is only about 3% of the product yield. Although the molar absorption of the different platinated trinucleotides might not be exactly the same, product ratios have been estimated on peak areas monitored by UV detection (254 nm).

To investigate both the temperature and the hydrolysis effect, excess GAG was incubated with the two *cis*-Pt solutions at several temperatures (30, 50, and 80 °C).

The results of the quantitative experiments clearly demonstrated that, within 2% experimental error, no differences are observed in the product ratio, owing to temperature and/or hydrolysis effects (not shown). Since oligonucleotides with purine bases usually exhibit temperature-dependent intramolecular-stacking effects, at least a small temperature effect on the product ratio was expected. Apparently, the effect of the trinucleotide conformation is far less important than the kinetic preference of *cis*-Pt for guanines. At this point, one has to note that the temperature effect in the "in vitro" study on the product ratio in the reaction of *cis*-Pt with DNA also appeared to be neglectible.[6]

Scheme I. Overview of the Two Steps of Interaction of *cis*-Pt with GAG[a]



[a] Relative amounts (%) are indicated.

Two explanations are possible for the observation that both solutions containing different hydrolysis species give the same product ratio. Either both aqua species, *cis*-$Pt(OH)(H_2O)(NH_3)_2^+$ and *cis*-$PtCl(H_2O)(NH_3)_2^+$, have the same kinetics toward the trinucleotide, or the reaction of the former compound with GAG is much faster than that of the latter compound, indicating that complete hydrolysis of the latter aqua species has to occur before the first platination step can take place. This latter possibility is supported by literature data.[30]

From the temperature and the hydrolysis experiments, we learn that in our case both parameters do not affect the product ratio.

Kinetic Aspects of the Chelate Formation. The formation of a bifunctional *cis*-Pt adduct with DNA is often considered as a two-step process.[32] In the first reaction step of *cis*-Pt with GAG, the platinum compound will react either with the 5′- or with the 3′-guanine, but not with the adenine, owing to the large preference of *cis*-Pt for guanine.[33] If the ratio of this first platination step (3′-binding vs. 5′-binding) is known, a more detailed insight in kinetic aspects of the subsequent chelate formation leading to both adducts would be obtained. To study the first binding step of the bifunctional *cis*-Pt, the monofunctional [PtCl(dien)]Cl is often used.

Equimolar amounts of GAG and [PtCl(dien)]Cl were allowed to react at pH 7.0 and 20 or 50 °C. The reactions resulted in both cases in two products, as was demonstrated by proton NMR spectra that were recorded immediately after the reaction. Quantitation of these products was performed by integration of the well-resolved H1′ signals. After separation of the products by means of gel filtration, NOE experiments were performed with the major product. A clear NOE effect from the irradiated H8 of the platinated guanine on the 3′-guanine (not shown) proved unambiguously the identity of this product as Pt(dien)[d-(GpApG)-*N*7(3)]. This, combined with the integration results, led to the conclusion that 35% of the platinum compound was bound to the 5′-guanine, resulting in Pt(dien)[d(GpApG)-*N*7(1)] (*G*AG) while 65% was coordinated to the 3′-guanine, resulting in Pt(dien)[d(GpApG)-*N*7(3)] (GA*G*). Also in this case, no significant differences were observed in these percentages between the reaction carried out at 20 and at 50 °C. The preference for the 3′-guanine can be explained by a directing effect of the 5′-connected phosphate, which is absent for the 5′-guanine.[34]

Combination of the data of the first platination step with the results for the ultimately formed chelates leads to Scheme I.

The scheme illustrates the large kinetic preference of *cis*-Pt for guanine. Although—from a stereochemical point of view—a chelate in which two neighboring purines are involved is more easily formed than a GNG chelate—as concluded from the cis-Pt interaction with pGpGpG[38]—this strong preference for guanines results in a considerable amount of *GAG*. Another interesting

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point is that only an AG chelate but no GA chelate is found, although the latter adduct has been reported in a dinucleotide study with d(GpA).[35] After the first reaction step of *cis*-Pt with GAG, the intermediate complex in which the platinum is bound to the 3'-guanine (see Scheme I) can react further with the 5'-guanine (about 70%; with a 45/65 ratio, 100%) or with the adenine (about 30%; with a 20/65 ratio, 100%). However, when *cis*-Pt is coordinated to the 5'-guanine after the first platination step, only *GAG* is ultimately formed. One has, however, to note[36] that—assuming a B-DNA like conformation for GAG—the proximity of the platinum atom, when monofunctionally bound to the 3'-guanine, to the adenine N7 is about 2 Å, roughly the usual Pt–N7 distance found in most Pt–nucleobase crystal structures. The platinum–adenine N7 distance is much larger when platinum is coordinated to the 5'-guanine. These geometrical considerations can nicely explain why only AG and no GA chelate is observed. Since the tetranucleotide d(GpAgGpA) adopts a B-DNA type structure in solution,[39] the assumption of a similar structure for GAG seems valid.

These findings agree well with results obtained from the earlier study in which *cis*-Pt adducts formed in DNA were identified,[6] as well as from a crystallographic study concerning *cis*-Pt-soaked $tRNA^{Phe}$ crystals.[36] In both cases, only AG and no GA chelation was observed. Apart from N7, N1 platination of adenine in modified nucleobases and dinucleotides has also been observed.[23,37] This binding mode is probably less interesting, since N1 sites of purines in double-stranded DNA are involved in Watson–Crick base pairing. In our study with a single-stranded trinucleotide offering the possibility of N1 binding, no indications for this chelation type were obtained.

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(39) Rinkel, L. J., personal communication.

Concluding Remarks. Summarizing the results of this study, it can be concluded that after the reaction of *cis*-Pt with the trinucleotide d(GpApG), two main products can be identified in a ratio that appears to be unaffected by the reaction temperature. Also no temperature effect can be observed for the first binding step with one of both terminal guanines, simulated by the binding of [PtCl(dien)]Cl. Partial or complete hydrolysis of *cis*-Pt, leading to different aquated *cis*-Pt species also has no effect on the product ratio.

We can conclude that the GAG sequence used in this trinucleotide offers a good model system to study kinetic aspects of *cis*-Pt interactions with oligonucleotides in a search for a better insight into the formation of the adducts of *cis*-Pt with DNA. However, we cannot translate the results described above immediately to larger DNA fragments. For that reason, studies have been started with larger oligonucleotides (both single and double stranded) that contain this sequence.

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Registry No. *cis*-$PtCl_2(NH_3)_2$, 15663-27-1; *cis*-$Pt(NH_3)_2$[d(GpApG)-*N7*(*1*),*N7*(*3*)], 105121-41-3; *cis*-$Pt(NH_3)_2$[d(GpApG)-*N7*(*2*),*N7*(*3*)], 105121-42-4; [PtCl(dien)]Cl, 14215-58-8; *cis*-Pt(OH)$(H_2O)(NH_3)_2^+$, 54933-51-6; Pt(dien)[d(GpApG)-*N7*(*3*)], 105121-43-5; Pt(dien)[d(GpApG)-*N7*(*1*)], 105121-44-6.

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Synthesis and Characterization of Tetra-N-alkylated Cyclam Ligands That Contain a Functionalized Nitrogen Substituent[1]

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Eight monofunctionalized, tetra-N-alkylated cyclam ligands have been prepared by derivatization of the secondary amine in trimethylcyclam (1,4,8-trimethyl-1,4,8,11-tetraazacyclotetradecane). Syntheses and properties of nickel(II) and/or copper(II) complexes of these ligands are given along with evidence for interconversion of diastereoisomers of the nickel and copper complexes of trimethylcyclam.

Introduction

There is an ever increasing number of applications for metal complexing agents that contain additional reactive functional groups. The presence of suitable functional groups can allow the binding of the complexing agent, or perhaps a complexed form, to biomolecules or to polymers and other solid supports. In the former case the ultimate application might be to bind a radioactive nuclide for either diagnostic or therapeutic purposes. In the latter case it might be to make a metal ion concentration device or to prepare a redox-active electrode coating. It may also be possible to prepare multinuclear metal complexes by coupling reactions involving these functional groups.

Our interest in this general area has been directed toward the synthesis of macrocyclic tetraamine ligands that have appended side chains containing functional groups. In principle, side chains can be attached at either carbon or nitrogen positions and they may be introduced before or after the cyclization reaction has been performed. The work described here deals with the synthesis of ligands **2**–**9** and their nickel and/or copper complexes.

Ligand **3**, its *N*,*N*-dimethyl analogue, and their Co(II), Ni(II), and Cu(II) complexes have been reported previously by Basak and Kaden.[2] Nickel, copper, and zinc complexes of **2** have been reported,[3] but preparative details and physical properties of the ligand have not. The syntheses of **2**–**9** utilize 1,4,8-trimethyl-

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